

EXPLORING THE GLYCOSYLATION MACHINERY OF ANIMAL MAMMARY GLANDS

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The ultimate requirements for any therapeutic glycoprotein are safety, efficacy, and consistency in therapeutic potential. For glycosylation this implies consistency across recombinant glycoprotein batches, with nonimmunogenic glycans that facilitate the required efficacy *in vivo*. As opposed to cell culture approaches, little is known about the glycosylation machinery of the mammary gland in the various animal species used for recombinant glycoprotein production. In view of the hormonal regulation of protein glycosylation throughout lactation, it is also important to investigate whether glycosylation of a transgenic glycoprotein remains consistent throughout lactation, even at the level of the individual animal.

Here, we report on the detailed glycosylation patterns of two biopharmaceutical glycoproteins, human C1 inhibitor and human acid α -glucosidase, produced in the milk of transgenic rabbits. Structural analysis of released glycans was carried out by a combination of mass spectrometry, NMR spectroscopy, and HPLC profiling. Both glycoproteins contained a broad array of N-glycans, made up of oligomannose-, hybrid-, and complex-type structures. Part of the complex-type structures are (α 1-6)-fucosylated at the innermost GlcNAc residue or (α 1-3)-fucosylated in the antennae (Lewis X). Remarkably, in both cases the oligomannose-type pattern indicates that besides the usual N-glycan processing route, also the alternative endo-mannosidase pathway is followed. Focusing on lactation parameters in the C1 inhibitor production system, it turned out that a decrease in the extent of sialylation, (α 1-6) core fucosylation, and oligomannose-type glycosylation with the progress of lactation and a positive correlation between expression level and oligomannose-type N-glycan content occurs. For the lysosomal enzyme acid α -glucosidase, GlcNAc(α 1-P-6) extensions of oligomannose-type structures, related with the lysosomal pathway, could be established, whereby it should be noted that all phosphate groups in the isolated glycoprotein were capped. With respect to the O-glycosylation pattern of the C1 inhibitor, only small core 1-type glycans were found with the usual (α 2-3)- and (α 2-6)-sialylations of O-glycoproteins of nonmucinous origin.